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THE UPTAKE AND METABOLISM OF DISSOLVED AMINO ACIDS BY BIVALVE LARVAE

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ABSTRACT

The rates of uptake and metabolism of ^{14}C-labeled glycine and alanine from sea water into larval oysters, *Crassostrea gigas* (Thunberg) and mussels, *Mytilus edulis* L. were determined. Kinetic studies showed that both species have a K_t value of 3–4 μM, indicating that bivalve larvae have amino acid transport mechanisms that function efficiently in natural sea water. The K_t values for larvae are similar to those reported for adult bivalves. However, larvae take up dissolved amino acids at approximately ten times the rate reported for adult bivalves on a gram dry weight basis. This difference in uptake capacity presumably reflects the greater absorptive surface area to volume ratio of a larva. Rates of metabolism of absorbed amino acids by larvae were also rapid. Following a 100 min exposure, oyster larvae incorporated 47% of the glycine into protein and 38% was produced as CO_2. In comparison to adults, larval bivalves have a more rapid weight-specific uptake and faster rate of utilizing absorbed amino acids. Dissolved nutrients may be of vital importance to larvae when particulate food is scarce since they are often provided with minimal food reserves by the parent.

INTRODUCTION

The possibility that dissolved organic material (DOM) in sea water may contribute to the nutrition of marine invertebrates is an attractive idea, since dissolved organic carbon (circa 3 mg C/l; Williams, 1975) is present at concentrations approximately ten times that of particulate organic carbon (circa 0.2 mg C/l; Parsons, 1975). During this century there have been many studies on the uptake of DOM directly from sea water by marine invertebrates (see reviews by Krogh, 1931; Jørgensen, 1976; Stewart, 1979; Stephens, 1981). Studies to date have demonstrated that ^{14}C-labeled substrates, such as amino acids and sugars, are removed from dilute solution in sea water by soft-bodied marine invertebrates and that uptake occurs across the body wall or via specialized organs such as the ctenidia of molluscs. The relationship between uptake and substrate concentration is saturable, indicating a carrier-mediated process typical of active transport systems.

Nearly all of the studies examining the role of DOM in animal nutrition have been carried out using adult invertebrates. However, there are some reports that DOM can be utilized by larval forms. For example, embryos of the ophiuroid *Amphipholis squamata* have a greater capacity for assimilating dissolved glycine and glucose into their tissues than do adult ophiuroids (Fontaine and Chia, 1968). Larvae of the polychaete worm *Nereis virens* absorb 200 times more amino acid per gram

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Abbreviations: DOM—dissolved organic material.

wet weight than adults (Bass *et al.*, 1969). Reish and Stephens (1969) carried out a detailed investigation on the relationship between body size and the rate of glycine uptake by the polychaete, *Neanthes arenacoedentata*. The linear relation between log uptake and log wet body weight suggested that uptake is dependent upon surface area. Significantly, these authors also found that non-feeding larval stages absorbed glycine at a rate 3–5 times that of feeding stages. Larvae of the bivalve *Mactra* sp. take up ^{14}C-glucose (Crane *et al.*, 1957) and larvae of the Pacific oyster *Crassostrea gigas* have been shown to take up an undefined ^{14}C-labeled algal exudate from sea water (Fankboner and deBurgh, 1978). Rice *et al.* (1980) demonstrated that larvae of the European oyster *Ostrea edulis* absorb dissolved amino acids.

Although there is evidence that bivalve larvae can take up DOM, the contribution of this pathway to their nutritional requirements remains obscure. Manahan and Crisp (1982) demonstrated that the velum of a bivalve larva is capable of absorbing dissolved amino acids directly from sea water. Since the velum presents a large surface area for absorption of dissolved nutrients, bivalve larvae are anatomically well adapted to benefit from this type of assimilation. In this report, I describe kinetic and time course studies on the rates of uptake and metabolism of ^{14}C-labeled amino acids by veligers and pediveligers of the Pacific oyster, *Crassostrea gigas* (Thunberg), and by veligers of the mussel, *Mytilus edulis* L.

Materials and Methods

Animals

The routine rearing of larvae was carried out as described by Loosanoff and Davis (1963). Two batches of oyster larvae were used. The *C. gigas* pediveligers were supplied by the Fisheries Experimental Station, Conway, North Wales, where they had been reared at 25°C and a salinity of 25‰. A second batch of *C. gigas* larvae was spawned and reared at Menai Bridge for 20 days (25°C; 32‰) until the larvae had reached the late veliger stage of 300 μm shell length. *M. edulis* larvae were grown at 15°C and 32‰ for 50 days; by then all the larvae had reached the eyed stage.

Incubation media

All glassware was sterilized either by autoclaving at 120°C for 20 min, or by heat-sterilization at 150°C overnight. Plastic sieves used to handle the larvae were pasteurized at 60°C overnight. For experiments, larvae were placed in sterile-filtered (0.2 μm Millipore) sea water at the appropriate temperature and salinity. The labeled amino acids L-(U-^{14}C) alanine (172 mCi/mmol) or (U-^{14}C) glycine (118 mCi/mmol) were added to give the required test concentrations.

Kinetic and time course experiments

Larvae used in uptake experiments were siphoned onto a 45 μm mesh screen to select for uniform size. The larvae were blotted to remove adherent fluid, washed three times with 100 ml of sterile-filtered sea water, and distributed into glass vials at a density of about 40/ml. Two experiments were carried out in parallel for each group of larvae, *C. gigas* pediveligers and *M. edulis* veligers. (a) For kinetic experiments, larvae were exposed for the shortest practicable period (5 min) to a ^{14}C-labeled amino acid ranging in concentration from 0.1 μ*M* to 10 μ*M*; (b) in time course experiments, the larvae were exposed to circa 0.5 μ*M* ^{14}C-labeled substrate for 100 min. Separate vials of larvae were harvested at 0, 10, 20, 40, 60, 80, and

100 min to measure the ^{14}C-label in the larvae. In parallel time course experiments, the production of $^{14}CO_2$ by oyster larvae was measured.

Measurement of $^{14}CO_2$ production

Actively swimming bivalve larvae spend periods of time with their vela close to the sea water/air interface. During this behavior, known as "rafting," any volatile constituent containing ^{14}C might pass directly from the larva into the air. Respired $^{14}CO_2$ could thus be underestimated in samples of medium taken at the end of the experiment. Therefore, a system was developed to trap all the $^{14}CO_2$ produced. Larvae were placed in a 2 ml glass vial which was glued to the base of a 15 ml glass vial. One ml of 10% KOH was pipetted onto the base of the 15 ml vial. The system was sealed by placing a teflon Suba Seal into the opening of the 15 ml vial. One such $^{14}CO_2$ trapping system was set up for each of the 21 determinations made during a 100 min time course experiment. At a given time interval, 0.5 ml of HCl was injected through the Suba Seal into the 2 ml vial containing the larvae, thereby stopping the experiment and increasing the acidity of the sea water to pH 1–2. Any $^{14}CO_2$ present was driven off and trapped in the 10% KOH during the following 16–24 h; thereafter, the vial was opened and the larvae in each one counted. Data could then be expressed as cpm of $^{14}CO_2$ produced per larva.

The efficiency of $^{14}CO_2$ trapping was determined using radioactive bicarbonate standards. A series of $^{14}CO_2$ trapping vials containing 1 ml of standard were set up and acidified. 0.5 ml samples of the initial 1 ml 10% KOH were taken from each vial at various time intervals. The KOH sample was placed in a scintillation vial containing 1 ml methanol and 10 ml Aquasol. The addition of methanol was important, as it prevented the formation of a precipitate when KOH was added directly to Aquasol. By 16–24 h, 95% of the $^{14}CO_2$ was trapped.

Determination of radioactivity in larvae

At each sampling in the time course experiment, larvae were poured from the vial onto a 45 μm mesh sieve and rinsed with 100 ml of sea water. In this condition, the larvae can be stored at 4°C on damp filter paper. Preliminary experiments showed that no decrease in radioactivity per larva occurred during a 5 h storage; in fact, not more than 2 h of storage was ever necessary. The sampling error (95% confidence limits/mean) for the 15 data points of these tests was 5–6% for both mussel and oyster larvae. Three samples of larvae from each sieve were placed on gridded Millipore filters (type: RAWG 02500) and each filter in turn placed on a glass slide and the larvae counted under a microscope. The larvae and filter were placed in a glass scintillation vial (teflon capped) and digested with 1 ml of Tissue Solubilizer (NCS, New England Nuclear) at 50°C overnight. Samples were then bleached to improve radioactive counting efficiency by incubation with 1 ml of 20% benzoyl peroxide in toluene at 50°C for 3 h. After cooling, 10 ml of Aquasol was added to each vial. All samples were stored in the dark for at least 16 h prior to counting. Quench correction was carried out by the addition of internal standards using ^{14}C-hexadecane.

The uniform procedure used to prepare samples for radioactive counting, reduced any differences in quenching caused by digesting different numbers of larvae per sample. Knowing the total radioactivity per sample, the number of larvae in that sample, and the specific activity of the isotope, uptake rates could be expressed as grams ^{14}C-labeled amino acid per larva.

TABLE I

Scheme for determining the percentage of ^{14}C in the lipid, protein, and small molecular weight (TCA soluble) fractions of bivalve larvae

Add 800 μl distilled H_2O to freeze dried sample		
Homogenate		
Lipid	*Protein*	*Total*
3 × 100 μl replicate samples:	3 × 100 μl replicate samples:	Single 100 μl sample:
100 μl sample + 100 μl H_2O + 250 μl $CHCl_3$ + 500 μl CH_3OH. Shake 1 min. Stand at 4°C 10 min. Centrifuge 8,000 *g* for 1 min. Removal 800 μl supernatant (cf. 950 added). 800 μl supernatant + 200 μl $CHCl_3$ + 200 μl H_2O Shake 1 min. Centrifuge 8,000 *g* 1 min. Remove top phase. Wash bottom phase ×3 with 500 μl Folch rgt. Dry lower phase at 54°C Residue = LIPID + 500 μl NCS Tissue Solubilizer. Digest at room temperature overnight.	100 μl sample + 100 μl H_2O + 100 μl 15% cold TCA. Shake 1 min. Stand at 4°C for 10 min. Centrifuge 8,000 *g* 1 min. Remove all of supernatant. Wash ppt. ×3 with 200 μl 5% TCA removing supernatant each time. ppt. = Protein + 500 μl NCS Tissue Solubilizer. Digest at room temperature overnight.	100 μl + 500 μl NCS Tissue Solubilizer. Digest overnight.

Biochemical fate of amino acid carbon

In the experiments determining radioactivity in larvae, there always remained a large surplus of larvae available for biochemical fractionation. These larvae were harvested at 10 and 100 min during the time course experiment described above, and were washed onto a GFC filter paper and freeze dried. Using methods modified from Holland and Gabbott (1971), homogenates of these larvae were quantitatively separated into lipids, proteins, and, by subtraction from the total, small molecular weight compounds and carbohydrates (TCA soluble, see Table I). After separation of the fractions, the amount of radioactivity in digests of the various fractions was determined. The total uptake of ^{14}C-labeled amino acid was measured by adding the observed ^{14}C incorporated in the tissue to the $^{14}CO_2$ expired. The former could be proportioned into that metabolized into protein, lipid, and TCA soluble compounds.

Oxygen consumption

The rate of O_2 consumption by *C. gigas* larvae (300 μm shell length) at 21°C and 25‰ was measured with a Radiometer oxygen electrode. The method is described in detail by Davenport (1976).

Results

Crassostrea gigas pediveligers

(a) Kinetics of glycine uptake

Figure 1 shows the effect of increasing substrate concentration on the rate of glycine uptake by *C. gigas* pediveligers. Glycine uptake is evidently concave to the

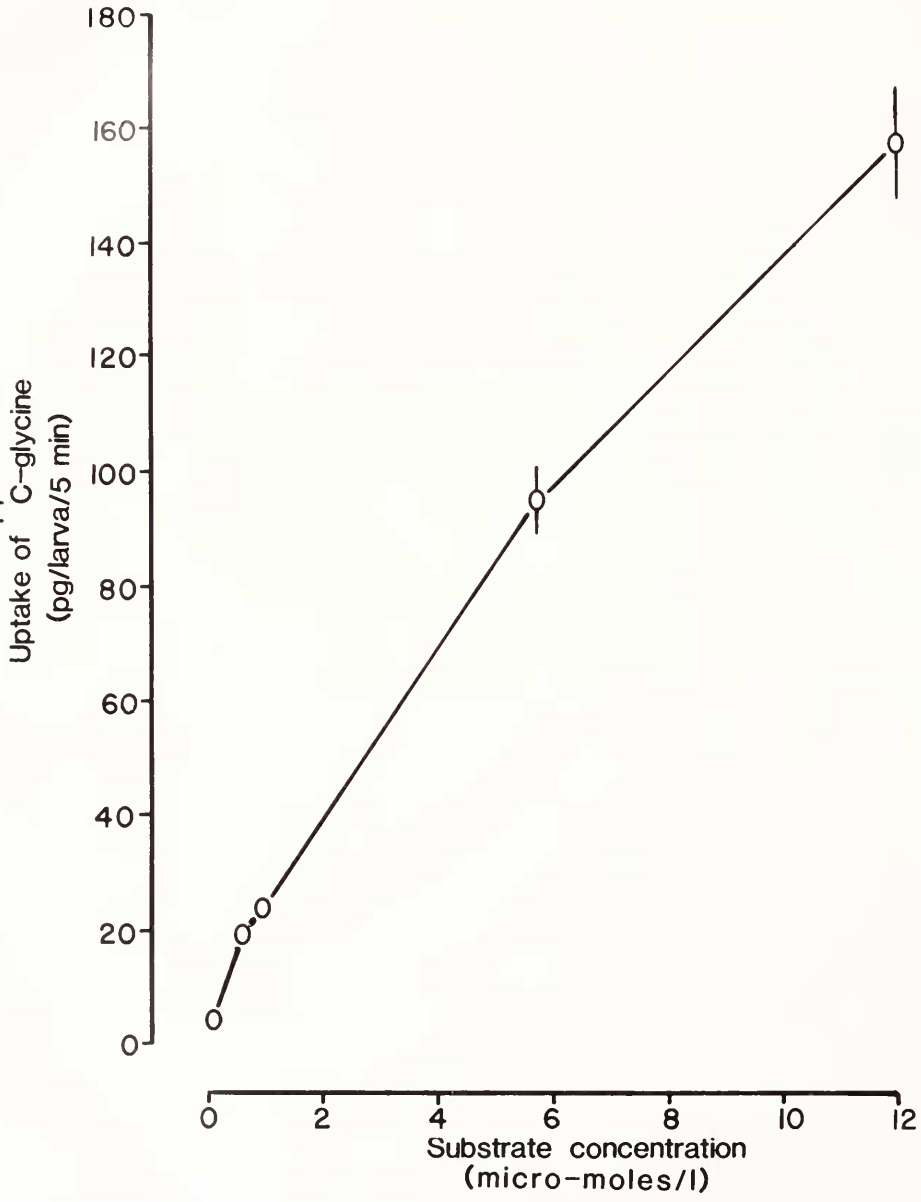

FIGURE 1. The uptake of ^{14}C-glycine by *C. gigas* pediveligers at substrate concentrations ranging from 0.1 μM to 10μM. Temperature = 25°C. Salinity = 25‰. Data points are mean ± S.E. for n = 3. Where no bar errors are shown on a data point, the bars lie within the graphical presentation of the point.

TABLE II

Kinetic constants for amino acid transport by oyster (C. gigas) *and mussel* (M. edulis) *larvae*

Species	Temp.	Salinity	Substrate	$K_t \pm$ S.E.	V_{max}
C. gigas	25°C	25‰	glycine	3.7 $\mu M \pm$ 1.0	1.8 ng larva^{-1} hr^{-1}
M. edulis	15°C	32‰	L-alanine	3.5 $\mu M \pm$ 0.5	0.9 ng larva^{-1} hr^{-1}

abscissa, and therefore appears to be saturable. A Michaelis-Menten analysis of uptake was made using Hofstee's (1959) linear transformation. The transport constant, K_t, is the substrate concentration at which uptake is experimentally determined to be half the maximum transport capacity of the system ($\frac{1}{2}$ V_{max}). Table II gives the kinetic data for *C. gigas* larvae. A low K_t of 3.7 μM was obtained for glycine transport.

(b) Time course of glycine metabolism

The time course of ^{14}C-glycine uptake from a concentration of 0.59 μM is shown in Figure 2. Since the numbers of larvae used were in the order of 40/ml, a calculation based on the uptake rates given below shows that total larval uptake would not reduce the substrate concentration by more than 0.02%. The glycine concentration therefore remained virtually constant during the experiment and the linearity of the plots in Figure 2 is to be expected. Regression of uptake in the larva on time gave a rate of 1.68 pg ^{14}C-glycine larva^{-1} min^{-1}; glycine appeared as $^{14}CO_2$ at a rate of 1.02 pg ^{14}C-glycine larva^{-1} min^{-1}. The striking feature of these data is the rapid rate of $^{14}CO_2$ production. A more complete picture of the fate of glycine after absorption by pediveligers is given in Table III. After 100 min exposure, 47% of the glycine carbon had been synthesized into protein and 38% appeared as $^{14}CO_2$. In this short time, less than 2% of the ^{14}C-carbon was found in lipid. As the exposure time to glycine was increased for 10 to 100 min, the amount of glycine metabolized by the larva to lipid, protein, and CO_2 continued to increase by about a factor of 10.

Crassostrea gigas veligers

Figure 3 shows the uptake of glycine by veligers from a concentration of 0.69 μM. $^{14}CO_2$ is also produced from glycine by veligers at a rapid rate of 0.74 pg larva^{-1} min^{-1}, when compared to an uptake rate into the larva of 1.22 pg ^{14}C-glycine larva^{-1} min^{-1}. Table IV gives the more detailed account of glycine metabolism by veligers. By 100 min, 16% of the ^{14}C-label was found in protein and 33% had been recovered as CO_2. Lipid contained less than 1%.

Mytilus edulis veligers

(a) Kinetics of alanine uptake

Figure 4 shows the kinetic response of *M. edulis* larvae to increasing alanine concentrations. The kinetic constants obtained from a Hofstee plot of these data are given in Table II. A low K_t for alanine of 3.5 μM was obtained, which is very similar to that of 3.7 μM obtained for glycine uptake by oyster larvae.

(b) Time course of alanine metabolism

The uptake of alanine from a concentration of 0.41 μM is shown in Figure 5. There are no data for $^{14}CO_2$ production during this experiment. Table V gives the

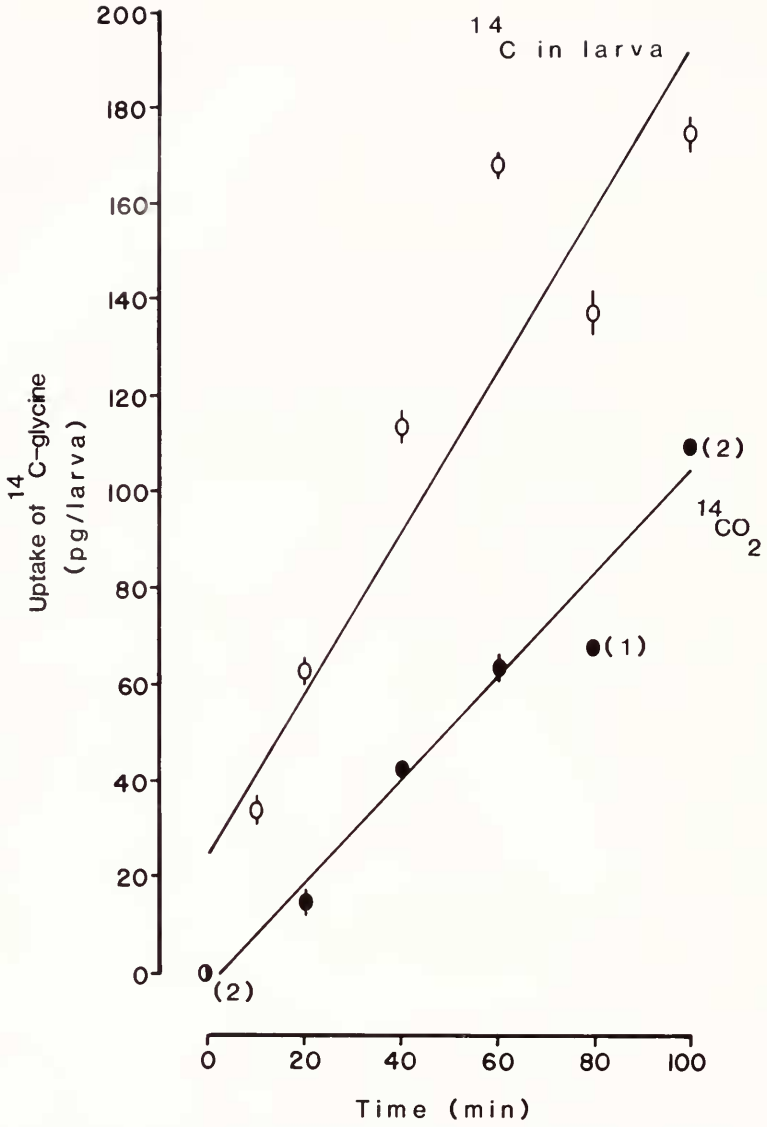

FIGURE 2. Time course of ^{14}C-glycine uptake and oxidation by *C. gigas* pediveligers. ^{14}C-glycine concentration = 0.59 μM. Temperature = 25°C. Salinity = 25‰. Open circles represent ^{14}C recovered in larvae. Closed circles represent $^{14}CO_2$ produced by larvae. All data points are mean ± S.E. for n = 3. Where n is less than 3, the number of replicates is given above the data point. Where no bar errors are shown on a data point, the bars lie within the graphical presentation of the point.

Regression equations

Treatment	*n*	*a*	*b* ± *S.E.*	r^2
Uptake by larva	21	24.10	1.68 ± 0.15	0.86
$^{14}CO_2$ production	17	−2.52	1.02 ± 0.07	0.98

TABLE III

The metabolism of ^{14}C-glycine by C. gigas *pediveligers*

Time		Metabolic fraction (pg ^{14}C-gly/larva)	Metabolic fraction (% of total)
10 min	Total uptake	42.3	
	Lipid	0.9	2.1
	Protein	16.5	39.0
	CO_2	8.4	19.9
	TCA soluble	16.5	39.0
100 min	Total uptake	283.0	
	Lipid	4.2	1.5
	Protein	133.6	47.2
	CO_2	109.0	38.5
	TCA soluble	36.2	12.8

biochemical fate of ^{14}C-alanine following assimilation. A similar pattern to that obtained for oyster larvae is evident. A greater percentage of the ^{14}C-label was present in protein than in lipid, and the amount of assimilated ^{14}C-alanine increases with exposure time.

Oxygen consumption of Crassostrea gigas veligers

The O_2 consumption of *C. gigas* larvae (300 μm) was determined at an early stage of the investigation to be 6.6 nl and 9.5 nl O_2 larva^{-1} h^{-1} for two replicate experiments carried out at 21°C. Assuming a Q_{10} of 2, this oxygen consumption would increase to 10.6 nl O_2 larva^{-1} h^{-1} at 25°C, the temperature of the glycine uptake experiments. This figure accords well with an estimate of 9–11 nl O_2 larva^{-1} h^{-1} for larvae of the European oyster *Ostrea edulis* at the same size (297 μm), based on the value quoted by Crisp (1976) of 5–6 ml O_2 (g dry wt)$^{-1}$ h^{-1}, and on Holland and Spencer's (1973) dry organic weight for a larva of this size (1.84 μg).

DISCUSSION

Rates of uptake of amino acids

The kinetic data for oyster and mussel larvae (Table II) include a K_t of 3–4 μM for each species. This low K_t indicates that bivalve larvae have amino acid transport mechanisms that function efficiently in natural sea water where the free amino acid concentrations lie in this order of magnitude (*e.g.*, North, 1975). The K_t values for the larvae are similar to those reported by Wright and Stephens (1978) for intact adult mussels, *Mytilus edulis* (K_t = 2–5 μM). Hence, both larval and adult bivalves are adapted to utilize dissolved amino acids in sea water.

However, an important difference in the rates of amino acid uptake can be shown by comparing alanine uptake by larvae and adults. Wright and Stephens (1978) report that a mussel weighing 5 g (wet wt minus shell) can accumulate amino acids from a micromolar solution at a rate of 2 μmoles/h at 20°C. Assuming 20% of the mussel's weight is dry organic weight, an adult could take up alanine at a rate of 178 μg alanine (g dry wt)$^{-1}$ h^{-1}. In this work, larvae of *M. edulis* were observed

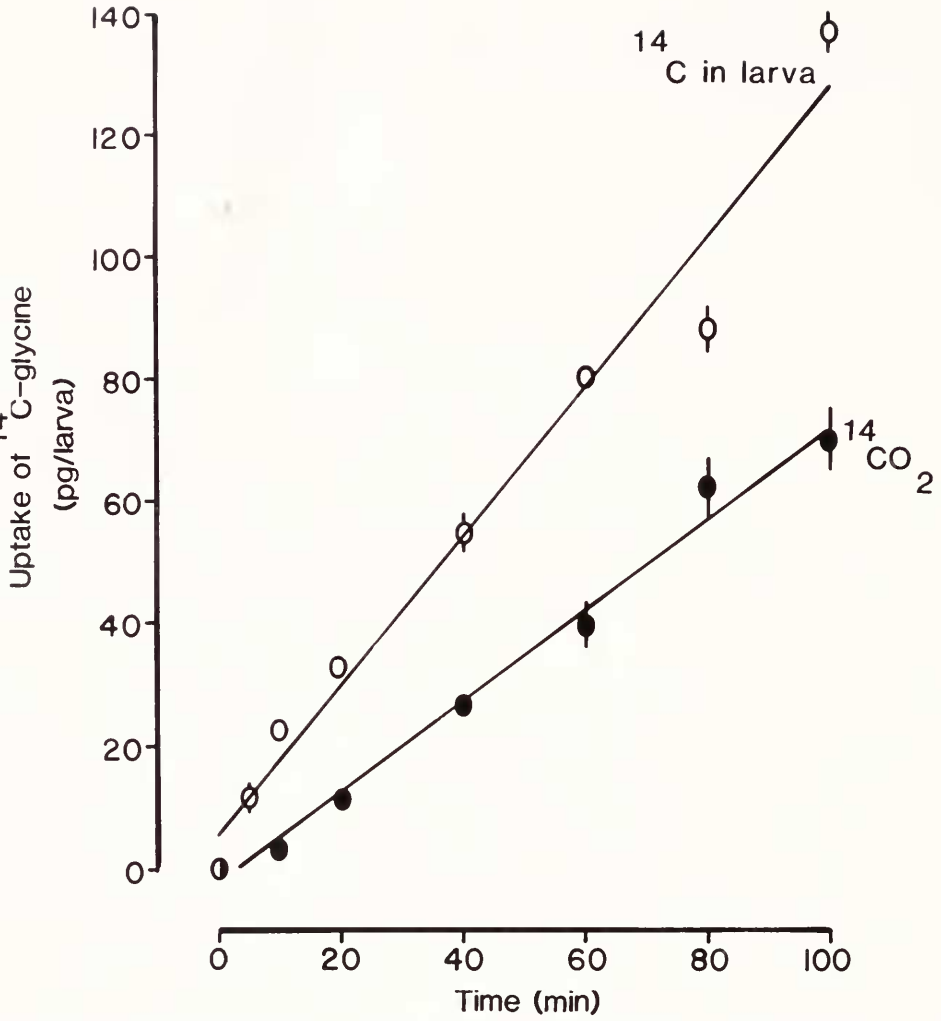

FIGURE 3. Time course of ^{14}C-glycine uptake and oxidation by *C. gigas* veligers. ^{14}C-glycine concentration = 0.69 μM. Temperature = 25°C. Salinity = 32‰. Open circles represent ^{14}C recovered in larvae. Closed circles represent $^{14}CO_2$ produced by larvae. All data points are mean ± S.E. for n = 3. Where no bar errors are shown on a data point, the bars lie within the graphical presentation of the point.

Regression equations

Treatment	*n*	*a*	*b* ± *S.E.*	r^2
Uptake by larva	24	5.48	1.22 ± 0.05	0.97
$^{14}CO_2$ production	21	−2.32	0.74 ± 0.03	0.96

to accumulate alanine from a micromolar solution at a rate of 180 pg larva^{-1} h^{-1} at 15°C. Crisp (1976) recalculated the dry organic weight of a *M. edulis* larvae as 0.2 μg from Zeuthen's (1947) values of nitrogen content. Taking this figure, a larva would accumulate 0.9 mg alanine (g dry wt)$^{-1}$ h^{-1} at 15°C. At 20°C, corresponding

TABLE IV

The metabolism of ^{14}C-glycine by C. gigas *veligers*

Time		Metabolic fraction (pg ^{14}C-gly/larva)	Metabolic fraction (% of total)
10 min	Total uptake	25.6	
	Lipid	0.1	0.004
	Protein	2.9	11.3
	CO_2	3.2	12.5
	TCA soluble	19.4	75.8
100 min	Total uptake	206.9	
	Lipid	1.1	0.5
	Protein	33.2	16.0
	CO_2	69.8	33.7
	TCA soluble	102.8	49.7

to Wright and Stephens' experiment, the rate would be higher. For example, assuming a Q_{10} of 2, a larva could take up 1272 μg alanine (g dry wt)$^{-1}$ h^{-1} at 20°C compared to a value of 178 μg alanine given above for the adult. Thus, on a relative weight basis, the larva is taking up alanine at a rate approximately one order of magnitude faster than that of the adult. Presumably this difference is a reflection of the greater absorption surface area to volume ratio of the larva.

Rates of metabolism of absorbed amino acids

Bamford and McCrea (1975) and Stewart and Bamford (1975) showed that in the adult cockle *Cerastoderma edule* and the clam *Mya arenaria* 95% of the labeled amino acid taken up remained unmetabolized after one hour of exposure, and in some cases it took up to 24 h for the major portion to be metabolized (*e.g.*, Stewart, 1977). The data presented here show that in larvae, the time scale is much shorter. For instance, Table III shows that *C. gigas* pediveligers metabolize at least 61% of the absorbed ^{14}C-glycine in just 10 min. Following 100 min exposure, only 12.8% of the ^{14}C-labeled glycine remained in the TCA soluble fraction, 87.2% of the ^{14}C appeared as lipid, protein, and $^{14}CO_2$.

The above studies show, not only that bivalve larvae have a transport system operating at a high affinity for absorbing amino acids at environmentally realistic concentrations, but also that, in comparison with adults, larvae have a more rapid weight-specific uptake, and a faster rate of metabolising these substrates.

Contribution of amino acid uptake to energy and growth requirements

Johannes *et al.* (1969) drew attention to the critical difference between an influx of radiolabeled substrate and a net entry of that substrate. Although the question of net flux has not been addressed in this study, Manahan *et al.* (1982) showed that adult mussels *M. edulis* are capable of net uptake of amino acids from concentrations as low as 38 n*M*. Recent work has shown a comparable picture with respect to net influx of amino acids in bacteria-free sea urchin larvae (Manahan *et al.*, 1983) and *C. gigas* larvae (unpublished data).

The uptake of dissolved organic nutrients by an organism has customarily been compared with it's respiration rate (*e.g.*, Stephens, 1964; Shick, 1975; Wright and

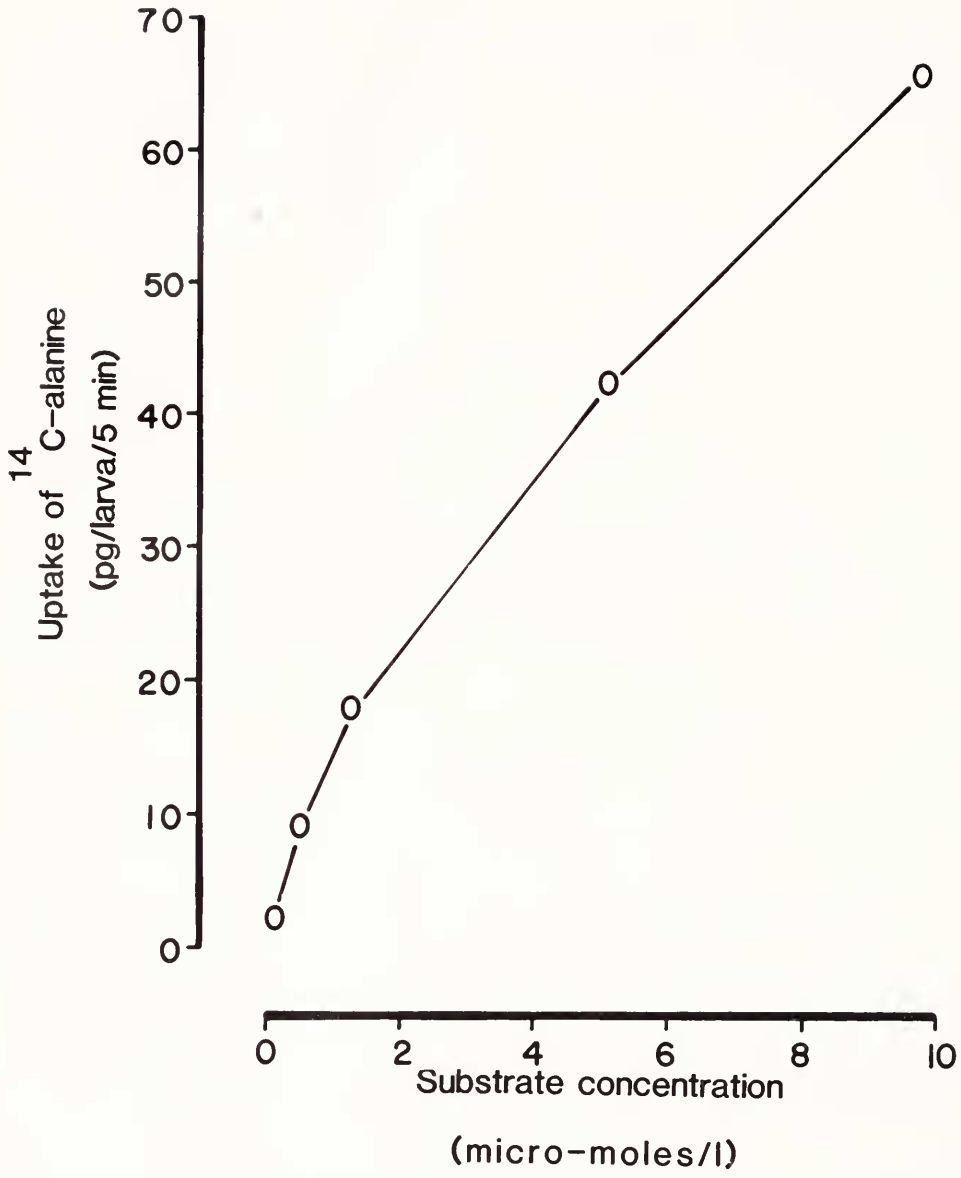

FIGURE 4. The uptake of ^{14}C-alanine by *M. edulis* veligers at substrate concentrations ranging from 0.1 μM to 10 μM. Temperature = 15°C. Salinity = 32‰. Data points are mean ± S.E. for n = 3. Where no bar errors are shown on a data point, the bars lie within the graphical presentation of the point.

Stephens, 1978). The rationale of this approach is that the dissolved organic substrates taken up can either themselves be oxidized, or can spare the animal's own energy reserves in providing energy for maintenance. This may be approximately true for full size adults without current reproductive expenditure, but it is manifestly inappropriate for growing larvae. The net growth efficiency, defined as rate of increase of biomass (or production, P) as a percentage of total energy requirements

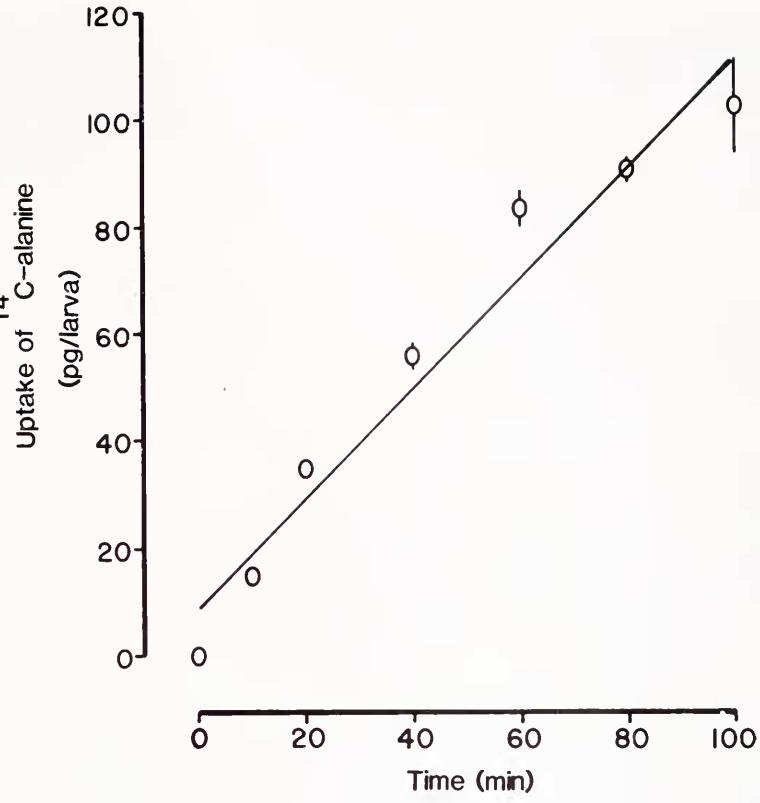

FIGURE 5. Time course of ^{14}C-alanine uptake by *M. edulis* veligers. ^{14}C-alanine concentration = 0.41 μM. Temperature = 15°C. Salinity = 32‰. Data points are mean ± S.E. for n = 3. Where no bar errors are shown on a data point, the bars lie within the graphical presentation of the point.

(P + respiration, R), is high in marine invertebrate larvae, usually about 70% (Holland, 1978), whereas in a hypothetical non-growing non-reproducing adult it would be zero. Therefore it is important to consider the energy cost of growth as well as respiration in larvae although not, of course, the cost of reproduction.

Data on cost of growth in developing pelagic larvae are scanty, and none exist for *C. gigas* veligers. Fortunately, comprehensive measurements have been made on the growth of the European oyster *Ostrea edulis* (Holland and Spencer, 1973), over the whole larval period including the late veliger stage. The latter stage corresponds in size, morphology, and linear growth rate with the 300 μm shell length *C. gigas* veligers used in this study and is therefore a very appropriate model. Table VI gives the rates of increase for each major biochemical component (Column I) for the 297 μ veliger of *O. edulis*. The total energy increase in the larva is computed as follows. Each component's (Column IV) daily percentage increase is multiplied by it's respective weight per larva (Column II) and by it's caloric content using values given by Crisp (1971). The results are the daily energy increases for each component, listed in Column V. These contributions are added to obtain the total figure of 22.86×10^{-4} cal larva^{-1} day^{-1}. To obtain the total energy requirements, the energy loss in respiration by *C. gigas* larvae must be added. The latter was obtained by multiplying the experimental value of 10 nl O_2 larva^{-1} h^{-1} (=240 nl

TABLE V

The metabolism of ^{14}C-alanine by M. edulis *veligers*

Time		Metabolic fraction (pg ^{14}C-ala/larva)	Metabolic fraction (% of total)
10 min	Total uptake	15.3	
	Lipid	0.2	1.3
	Protein	2.1	13.7
	CO_2	—	—
	TCA soluble	13.0	85.0
100 min	Total uptake	102.7	
	Lipid	1.0	1.0
	Protein	21.1	20.5
	CO_2	—	—
	TCA soluble	80.6	78.5

larva^{-1} day^{-1}) by the accepted oxycalorific equivalent of 4.8 Kcal/l O_2. Thus, the total energy requirement arrived at for *C. gigas* is 34.38×10^{-4} cal larva^{-1} day^{-1}.

The energy contributed by uptake of dissolved amino acids will depend on the ambient concentrations to which larvae are exposed. These are likely to range from 0.6 μM for surface waters to 6.0 μM for sediment waters (Henrichs and Farrington, 1979). The latter concentration may be relevant as larvae approach settlement. The rate of glycine uptake at these concentrations is 0.26 ng and 1.2 ng larva^{-1} h^{-1} (Fig. 1). Glycine has a molar heat of combustion of 233 Kcal/mole, giving 0.19×10^{-4} and 0.87×10^{-4} cal larva^{-1} day^{-1}, respectively. The uptake of glycine at these two concentrations would represent 0.6% to 2.8% of the total energy required by the larva. As a percentage of the energy respired (11.52×10^{-4} cal larva^{-1} day^{-1}), the figures would clearly be larger, viz. 1.6% to 7.5%.

The other important aspect of uptake of dissolved nutrients is their contribution of material for growth. In the *C. gigas* pediveliger the percentage of glycine taken

TABLE VI

Energy requirements of an oyster larva

I Component	II Wt/larva (μg)	III % in 12 day-old fed larva (297 μm)	IV Percentage increase/day	V Energy required (cal larva^{-1} day^{-1})
Protein Polysaccharide Free reducing substances RNA	1.09 0.20	59% 11%	14% 16%	8.62×10^{-4} 1.31×10^{-4}
Phospholipid	0.13	7%	18%	2.21×10^{-4}
Neutral lipid	0.42	23%	27%	10.72×10^{-4}
Total organic	1.84	100%	15%	22.86×10^{-4}
Shell	4.99	—	12%	—
Total larva	6.83	—	14%	—
Energy for respiration				11.52×10^{-4}
				$=34.38 \times 10^{-4}$

Compiled from Holland and Spencer, 1973.

up which is incorporated into protein is 39% and 47% following a 10 min and 100 min exposure, respectively. Apart from loss as $^{14}CO_2$, much of the remaining ^{14}C-label may also be eventually utilized in tissue synthesis. A conservative estimate of 50% contribution to protein synthesis therefore seems reasonable. Hence, at the uptake rate from 0.6 μM and 6.0 μM glycine, the amount of amino acid going into protein would be 3.1 ng and 14.4 ng larva^{-1} day^{-1}. Comparing this with the estimated daily rate of protein synthesis (Table VI) of 1.09 μg multiplied by 14% = 152 ng larva^{-1} day^{-1}, the percentage contribution at the two concentrations tested would be 2.0% and 9.5%.

It will be noted that the contribution of absorbed glycine to total energy is distinctly less than that to growth as measured in terms of protein synthesis. This is to be expected since glycine has a very low caloric content (3.1 Kcal/g) compared with the main energy stores of protein (5.65 Kcal/g) and lipid (9.45 Kcal/g) characteristic of bivalve and other marine invertebrate larvae (Holland, 1978). Although the estimated contribution from amino acids at naturally occurring concentrations appears small, sea water also contains other organic compounds which are available for uptake (Williams, 1975; Wangersky, 1978). Marine invertebrates are capable of taking up these compounds from micromolar concentrations, and the evidence in favor of a significant role for this alternative nutritional pathway is now considerable (see review by Stephens, 1981). Furthermore, during periods when particulate food is scarce, this supplementary mode of nutrition may be vital for pelagic larvae which are frequently on critical energy budgets (Crisp, 1974).

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LITERATURE CITED

Bamford, R. D., and S. R. McCrea. 1975. The active absorption of neutral and basic amino acids by the gill of the common cockle, *Cerastoderma edule*. *Comp. Biochem. Physiol.* **50A:** 811–817.

Bass, N., G. Chapman, and J. H. Chapman. 1969. Uptake of leucine by larvae and adults of *Nereis*. *Nature* **211:** 476–477.

Crane, R. K., A. K. Keltch, C. P. Waters, and G. H. A. Clowes. 1957. Preliminary studies on the incorporation of glucose-U-C^{14} into the polysaccharide of *Arbacia* and *Mactra* and its inhibition by 4,6-dimitro-o-cresol. *Biol. Bull.* **133:** 343 (Abstract).

Crisp, D. J. 1971. Energy flow measurements. Pp. 197–279 in *Methods for the study of Marine Benthos*. N. A. Holme and A. D. McIntyre, eds. Blackwell Sci. Publishing, Oxford.

Crisp, D. J. 1974. Energy relations of marine invertebrate larvae. *Thalassia Jugosl.* **10:** 103–120.

Crisp, D. J. 1976. The role of the pelagic larva. Pp. 145–155 in *Perspectives in experimental biology*. Vol. I. P. Spencer Davis, ed. Pergamon Press, Oxford and New York.

Davenport, J. 1976. A technique for the measurement of oxygen consumption in small aquatic organisms. *Lab. Pract.* **25:** 693–695.

Fankboner, P. V., and M. E. deBurgh. 1978. Comparative rates of dissolved organic carbon accumulation by juveniles and pediveligers of the Japanese oyster, *Crassostrea gigas* (Thunberg). *Aquaculture* **13:** 205–212.

Fontaine, A. R., and F. S. Chia. 1968. Echinoderms: an autoradiographic study of assimilation of dissolved organic molecules. *Science* **161:** 153–155.

Henrichs, S. M., and J. W. Farrington. 1979. Amino acids in interstitial waters of marine sediments. *Nature* **297:** 955–959.

Hofstee, B. H. J. 1959. Non-inverted versus inverted plots in enzyme kinetics. *Nature* **184:** 318–326.

Holland, D. L., and P. A. Gabbott. 1971. A microanalytical scheme for the determination of protein, carbohydrate, lipid and RNA levels in marine invertebrate larvae. *J. Mar. Biol. Assoc. U. K.* **51:** 659–668.

HOLLAND, D. L., AND B. E. SPENCER. 1973. Biochemical changes in fed and starved oysters *Ostrea edulis* (L) during larval development, metamorphosis and early spat growth. *J. Mar. Biol. Assoc. U. K.* **53:** 287–298.

HOLLAND, D. L. 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. Pp. 85–119 in *Biochemical and Biophysical Perspectives in Marine Biology.* Vol. IV. D. C. Malins and J. R. Sargent, eds. Academic Press, London.

JOHANNES, R. E., S. L. COWARD, AND K. L. WEBB. 1969. Are dissolved amino acids an energy source for marine invertebrates? *Comp. Biochem. Physiol.* **29:** 283–288.

JORGENSEN, C. B. 1976. August Pütter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. *Biol. Rev.* **51:** 291–328.

KROGH, A. 1931. Dissolved substances as food of aquatic organisms. *Biol. Rev.* **6:** 412–442.

LOOSANOFF, V. L., AND H. C. DAVIS. 1963. Rearing of bivalve molluscs. *Adv. Mar. Biol.* **1:** 1–136.

MANAHAN, D. T., AND D. J. CRISP. 1982. The role of dissolved organic material in the nutrition of pelagic larvae: Amino acid uptake by bivalve veligers. *Am. Zool.* **22:** 635–646.

MANAHAN, D. T., J. P. DAVIS, AND G. C. STEPHENS. 1983. Bacteria-free sea urchin larvae take up neutral amino acids selectively from seawater. *Science* (in press).

MANAHAN, D. T., S. H. WRIGHT, G. C. STEPHENS, AND M. A. RICE. 1982. Transport of dissolved amino acids by the mussel, *Mytilus edulis:* Demonstration of net uptake from natural seawater. *Science* **215:** 1253–1255.

NORTH, B. B. 1975. Primary amines in California coastal waters: utilization by phytoplankton. *Limnol. Oceanogr.* **20:** 20–27.

PARSONS, T. R. 1975. Particulate organic carbonin the sea. Pp. 365–383 in *Chemical Oceanography.* Vol. II. J. P. Riley and G. Skirrow, eds. Academic Press, London.

REISH, D. J., AND G. C. STEPHENS. 1969. Uptake of organic material by aquatic invertebrates. V. The influence of age on the uptake of glycine-C^{14} by the polychaete *Neanthes arenaceodentata. Mar. Biol.* **3:** 352–355.

RICE, M. A., K. WALLIS, AND G. C. STEPHENS. 1980. Influx and net flux of amino acids into larval and juvenile European flat oysters, *Ostrea edulis* (L.). *J. Exp. Mar. Biol. Ecol.* **48:** 51–59.

SHICK, J. M. 1975. Uptake and utilization of dissolved glycine by *Aurelia aurita* scyphistomae: temperature effects on the uptake process: nutritional role of dissolved amino acids. *Biol. Bull.* **148:** 117–140.

STEPHENS, G. C. 1964. Uptake of organic material by aquatic invertebrates. III. Uptake of glycine by brackish water annelids. *Biol. Bull.* **126:** 150–162.

STEPHENS, G. C. 1981. The trophic role of dissolved organic material. Pp. 271–291 in *Analysis of Marine Ecosystems.* A. L. Longhurst, ed., Academic Press, London.

STEWART, M. G., AND D. R. BAMFORD. 1975. Kinetics of alanine uptake by the gills of the soft shelled clam, *Mya arenaria. Comp. Biochem. Physiol.* **52A:** 67–74.

STEWART, M. G. 1977. The uptake and utilization of dissolved amino acids by the bivalve *Mya arenaria.* Pp. 145–155 in *Twelfth European Marine Biology Symposium.* D. S. McLusky and A. J. Perry, eds. Pergammon Press, Oxford and New York.

STEWART, M. G. 1979. Absorption of dissolved organic nutrients by marine invertebrates. *Ann. Rev. Oceanogr. Mar. Biol.* **17:** 163–192.

WANGERSKY, P. J. 1978. Production of dissolved organic matter. Pp. 115–220 in *Mar. Ecol.* Vol. IV. O. Kinne, ed. Wiley, New York.

WILLIAMS, P. J. LE B. 1975. Biological and chemical aspects of dissolved organic material in seawater. Pp. 301–363 in *Chemical Oceanography.* Vol. II. J. P. Riley and G. Skirrow, eds. Academic Press, London.

WRIGHT, S. H., AND G. C. STEPHENS. 1978. Removal of amino acid during a single pass of water across the gill of marine mussels. *J. Exp. Zool.* **205:** 337–352.

ZEUTHEN, E. 1947. Body size and metabolic weight in the animal kingdom with special regard to the marine microfauna. *C. R. Trav. Lab. Carlsbrg Ser. Chim.* **26:** 161 pp.